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LS: Entry 1 of 1

File: DWPI

Oct 3, 1996

DERWENT-ACC-NO: 1996-455379

DERWENT-WEEK: 200029

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TITLE: Adenovirus vectors, partic. for gene therapy - contg. nucleotide sequences to prevent generation of replication-competent adenoviruses

Basic Abstract Text (1):

Nucleotide sequence (NS), which contains elements of an adenovirus (Ad) genome and a heterologous mammalian gene under the control of a eukaryotic transcriptional promoter, is capable of functioning as a vector for the expression of the heterologous gene in a cell of an individual, and is further characterised by: (a) absence of a 1st element of the Ad genome that is essential to replication or packaging of the Ad in a host mammalian cell, and placement in the NS at, or directly adjacent to, the location in the NS otherwise accepted by the 1st element, of a 2nd element of Ad genome that is itself essential to the replication or packaging of Ad in a host mammalian cell; (b) absence of the Ela-E1b region of the Ad genome, and placement of a stuffer sequence in the N5 in a region other than that of the heterologous gene, the vector being further characterised in that legitimate recombination of the sequence with an element that is present in a helper cell used to replicate or package the sequence, or with an element that is present in a cell of an individual, and having homology with the Ela-E1b region, leads to the prodn. of a lengthened NS that is less efficient than the unmodified NS at being packaged in the helper cell or in a cell of the individual; (c) absence of the Ela-E1b region of the Ad genome, and repositioning of the gene that encodes protein IX to a location that deviates from its normal location in the wild-type Ad genome; (d) absence of the Ela-E1b region of the Ad genome, and the protein IX region of the Ad genome and a sequence size that does not exceed 90 % of the length of the Ad genome; or (e) absence of the Ela-E1b region of the Ad genome, and the E4 region of the Ad genome except for the ORF6 region.

Equivalent Abstract Text (1):

Nucleotide sequence (NS), which contains elements of an adenovirus (Ad) genome and a heterologous mammalian gene under the control of a eukaryotic transcriptional promoter, is capable of functioning as a vector for the expression of the heterologous gene in a cell of an individual, and is further characterised by: (a) absence of a 1st element of the Ad genome that is essential to replication or packaging of the Ad in a host mammalian cell, and placement in the NS at, or directly adjacent to, the location in the NS otherwise accepted by the 1st element, of a 2nd element of Ad genome that is itself essential to the replication or packaging of Ad in a host mammalian cell; (b) absence of the Ela-E1b region of the Ad genome, and placement of a stuffer sequence in the N5 in a region other than that of the heterologous gene, the vector being further characterised in that legitimate recombination of the sequence with an element that is present in a helper cell used to replicate or package the sequence, or with an element that is present in a cell of an individual, and having homology with the Ela-E1b region, leads to the prodn. of a lengthened NS that is less efficient than the unmodified NS at being packaged in the helper cell or in a cell of the individual; (c) absence of the Ela-E1b region of the Ad genome, and repositioning of the gene that encodes

protein IX to a location that deviates from its normal location in the wild-type Ad genome; (d) absence of the Ela-E1b region of the Ad genome, and the protein IX region of the Ad genome and a sequence size that does not exceed 90 % of the length of the Ad genome; or (e) absence of the Ela-E1b region of the Ad genome, and the E4 region of the Ad genome except for the ORF6 region.

Equivalent Abstract Text (4):

Nucleotide sequence (NS), which contains elements of an adenovirus (Ad) genome and a heterologous mammalian gene under the control of a eukaryotic transcriptional promoter, is capable of functioning as a vector for the expression of the heterologous gene in a cell of an individual, and is further characterised by: (a) absence of a 1st element of the Ad genome that is essential to replication or packaging of the Ad in a host mammalian cell, and placement in the NS at, or directly adjacent to, the location in the NS otherwise accepted by the 1st element, of a 2nd element of Ad genome that is itself essential to the replication or packaging of Ad in a host mammalian cell; (b) absence of the Ela-E1b region of the Ad genome, and placement of a stuffer sequence in the N5 in a region other than that of the heterologous gene, the vector being further characterised in that legitimate recombination of the sequence with an element that is present in a helper cell used to replicate or package the sequence, or with an element that is present in a cell of an individual, and having homology with the Ela-E1b region, leads to the prodn. of a lengthened NS that is less efficient than the unmodified NS at being packaged in the helper cell or in a cell of the individual; (c) absence of the Ela-E1b region of the Ad genome, and repositioning of the gene that encodes protein IX to a location that deviates from its normal location in the wild-type Ad genome; (d) absence of the Ela-E1b region of the Ad genome, and the protein IX region of the Ad genome and a sequence size that does not exceed 90 % of the length of the Ad genome; or (e) absence of the Ela-E1b region of the Ad genome, and the E4 region of the Ad genome except for the ORF6 region.

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L6: Entry 2 of 4

File: PGPB

Nov 14, 2002

DOCUMENT-IDENTIFIER: US 20020168342 A1

TITLE: Novel adenoviral vectors, packaging cell lines, recombinant adenoviruses and methods

Detail Description Paragraph:

[0045] The present invention further provides the production of novel mutant viruses (particularly, adenoviruses and AAV), and novel recombinant adenoviruses and AAV (also referred to herein as recombinant adenoviral-derived and AAV-derived vectors) containing a transgene which will be expressed in the target cells. The recombinant adenoviral-derived and AAV-viral vectors are prepared using the packaging cell lines described above which comprise one or more distinct nucleotide sequences capable of complementing the part of the adenovirus or AAV genome that is essential for the virus' replication and which is not present in the novel recombinant adenoviral-derived and AAV-derived vectors. Recombinant adenoviral-derived and AAV-derived vectors will no longer contain genes required for the virus replication in infected target cells. More particularly, the recombinant adenoviral vectors will only contain the minimum essential cis-elements (i.e., ITRs and packaging signal sequence) and protein IX sequence, and be free of the E1 (specifically, E1a and E1b) and E4 regions, and may additionally be free of E3 and E2A regions and the viral structural genes. In the case of the recombinant AAV vectors, these vectors will contain deletions of the AAV virus Rep protein coding region or will only contain the minimal essential cis-elements. The latter will be generated from the AAV packaging cell line which contains the E1a, E1b, E2A and E4 gene regions, and the DNA encoding virus-associated RNA by co-transfected a non-packaging complementing AAV plasmid which is defective for packaging but supplies the wild type AAV gene products [Samulski, et al, (1987)].

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L6: Entry 3 of 4

File: USPT

Feb 16, 1999

DOCUMENT-IDENTIFIER: US 5872005 A

TITLE: Packaging cell lines for adeno-associated viral vectors

Detailed Description Text (13):

The present invention further provides the production of novel mutant viruses (particularly, adenoviruses and AAV), and novel recombinant adenoviruses and AAV (also referred to herein as recombinant adenoviral-derived and AAV-derived vectors) containing a transgene which will be expressed in the target cells. The recombinant adenoviral-derived and AAV-viral vectors are prepared using the packaging cell lines described above which comprise one or more distinct nucleotide sequences capable of complementing the part of the adenovirus or AAV genome that is essential for the virus' replication and which is not present in the novel recombinant adenoviral-derived and AAV-derived vectors. Recombinant adenoviral-derived and AAV-derived vectors will no longer contain genes required for the virus replication in infected target cells. More particularly, the recombinant adenoviral vectors will only contain the minimum essential cis-elements (i.e., ITRs and packaging signal sequence) and protein IX sequence, and be free of the E1 (specifically, Ela and Elb) and E4 regions, and may additionally be free of E3 and E2A regions and the viral structural genes. In the case of the recombinant AAV vectors, these vectors will contain deletions of the AAV virus Rep protein coding region or will only contain the minimal essential cis-elements. The latter will be generated from the AAV packaging cell line which contains the Ela, Elb, E2A and E4 gene regions, and the DNA encoding virus-associated RNA by co-transfected a non-packaging complementing AAV plasmid which is defective for packaging but supplies the wild type AAV gene products [Samulski, et al, (1987)].

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L7: Entry 6 of 6

File: USPT

Jan 13, 1998

DOCUMENT-IDENTIFIER: US 5707618 A

TITLE: Adenovirus vectors for gene therapy

CLAIMS:

1. A recombinant adenovirus vector having a deleted E1 region of the adenovirus genome, into which a heterologous gene has been inserted, and in which the protein IX gene has been relocated in the adenovirus genome to a location thereof other than the location in which said protein IX gene normally resides, such that generation of replication-competent adenoviruses is minimized or eliminated.

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L9: Entry 4 of 28

File: PGPB

May 15, 2003

DOCUMENT-IDENTIFIER: US 20030091534 A1

TITLE: Adenoviral vectors having a protein IX deletion

Detail Description Paragraph:

[0072] Plasmid pAd/MLP/p53/E1b- was used as the starting material for these manipulations. This plasmid is based on the pBR322 derivative pML2 (pBR322 deleted for base pairs 1140 to 2490) and contains adenovirus type 5 sequences extending from base pair 1 to base pair 5788 except that it is deleted for adenovirus type 5 base pairs 357 to 3327. At the site of the Ad5 357/3327 deletion a transcriptional unit is inserted which is comprised of the adenovirus type 2 major late promoter, the adenovirus type 2 tripartite leader cDNA and the human p53 cDNA. It is a typical E1 replacement vector deleted for the Ad5 E1a and E1b genes but containing the Ad5 protein IX gene (for review of Adenovirus vectors see: Graham and Prevec (1992)). Ad2 DNA was obtained from Gibco BRL. Restriction endonucleases and T4 DNA ligase were obtained from New England Biolabs. E. coli DH5.alpha. competent cells were purchased from Gibco BRL and 293 cells were obtained from the American Type Culture Collection (ATCC). Prep-A-Gene DNA purification resin was obtained from BioRad. LB broth bacterial growth medium was obtained from Difco. Qiagen DNA purification columns were obtained from Qiagen, Inc. Ad5 dl327 was obtained from R. J. Schneider, NYU. The MBS DNA transfection kit was purchased from Stratagene.

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Gly	Lys	Glu	Pro	Gly	Gly	Ser	Arg	Ala	His	Ser	Ser	His	Leu	Lys	Ser
405									410					415	
Lys	Lys	Gly	Gln	Ser	Thr	Ser	Arg	His	Lys	Lys	Leu	Met	Phe	Lys	Thr
			420					425				430			
Glu	Gly	Pro	Asp	Ser	Asp	Xaa									
						435									

What is claimed is:

1. A composition comprising a recombinant adenovirus expression vector and a pharmaceutically acceptable carrier, the vector comprising:
 - (a) an insert of exogenous DNA comprising a gene encoding a foreign protein; and
 - (b) adenovirus DNA in which all of the coding sequences of E1a, E1b, and protein IX, and at least part of E3 have been deleted.
 2. The composition of claim 1, wherein the protein IX polyadenylation site is deleted from the adenovirus vector.
 3. The composition of claim 1, wherein the adenovirus is a group C adenovirus selected from a serotype 1, 2, 5, or 6.
 4. The composition of claim 1, wherein the insert of exogenous DNA is up to 2.6 KB.
 5. The composition of claim 1, wherein the insert of exogenous DNA is up to 4.5 KB.
 6. The composition of claim 1, wherein the foreign protein is a functional protein or a biologically active fragment thereof.
 7. The composition of claim 1, wherein the foreign protein is a tumor suppressor protein.
 8. The composition of claim 1, wherein the foreign protein is a suicide protein or functional equivalent thereof.
 9. The composition of claim 1, wherein the gene encoding the foreign protein is expressed under control of a cytomegalovirus (CMV) promoter.
10. The composition of claim 1, wherein the gene encoding the foreign protein is expressed under control of an adenovirus promoter.
- 15 11. The composition of claim 1, wherein the exogenous DNA further comprises a polyadenylation site.
12. The composition of claim 1, wherein the vector is A/C/N/53.
- 20 13. The composition of claim 1, wherein the vector is A/M/N/53.
14. The composition of claim 1, further comprising a host cell transformed with the adenovirus vector.
15. The composition of claim 1, wherein the pharmaceutically acceptable carrier comprises a physiologically acceptable compound.
16. The composition of claim 7, wherein the tumor suppressor protein is p53.
- 30 17. The composition of claim 7, wherein the tumor suppressor protein is RB56.
18. The composition of claim 9, wherein the CMV promoter is the CMV immediate early promoter.
- 35 19. The composition of claim 18, wherein the adenovirus promoter is the adenovirus 2 major late promoter.

* * * * *